Cancer Metastasis

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Analysis of circulating tumor cells to dissect the biology of human cancer metastasis

More than 90% of cancer-related deaths, corresponding to more than eight million people worldwide each year, are due to the development of a metastatic disease. Clearly, these numbers reflect our limited understanding of the key processes that drive human cancer metastasis, and the need to develop new therapies that suppress the spread of cancer.

Several unsolved questions frame the metastasis research field, including the search of those molecular events that are fundamental for the metastatic process, and that would represent exceptional therapeutic targets. Cancer cells that leave the primary tumor site and are transported through the circulation to distant organs are referred to as circulating tumor cells (CTCs). CTCs are used as a noninvasive source of cancer cells for analysis of tumor genotypes (i.e. so-called liquid biopsy), yet their characterization is also an exceptional opportunity to dissect the biology of blood-borne metastasis. While CTCs are extraordinarily rare in circulation, even in patients with metastatic cancer (approximately one cancer cell among a billion normal blood cells), their isolation is highly dependent upon technological constraints. However, remarkable advances in the microfluidics field have now enabled the isolation of viable CTCs from virtually all cancer types, revealing highly unexpected features of the metastatic process.

For example, while the majority of CTCs circulate as single cells, they can also be found as clusters of 2–50 cells (a.k.a. CTC-clusters), with the ratio of single *versus* clustered CTCs varying significantly among different patients, and along disease progression. While CTC-clusters have been previously observed in human specimens, their role in the metastatic process was unknown. When combining microfluidic technologies for CTC isolation, single cell resolution RNA sequencing, patient samples and mouse models, we recently demonstrated that CTC-clusters represent key players in the metastatic process. First, we understood that the presence of CTC-clusters in the bloodstream of patients with breast and prostate cancer is associated with a shorter metastasis-free survival and overall survival, respectively, compared to patients in whom only single CTCs are found. Second, adopting multicolor mouse models to trace metastatic cancer cells *in vivo*, we concluded that CTC-clusters are oligoclonal units derived from the primary tumor (as opposed to be derived from intravascular aggregation events or the progeny of a single CTC), and that they are up to 50-fold more metastatic than single CTCs. Third, with a single cell-resolution RNA sequencing approach applied to human CTC-clusters and matched single CTCs from individual patients, we identified the cell-cell junction component plakoglobin to be required for CTC-clustering and metastasis. Together, these results highlight CTC-clusters as a previously unappreciated, yet potentially targetable mechanism of cancer dissemination.

Our research is now focused on the identification of the key vulnerabilities of CTC-clusters. In collaboration with Prof. Christoph Rochlitz, Prof. Viola Heinzelmann Prof. Alfred Zippelius and Prof. Walter Weber at the University Hospital Basel we routinely isolate CTCs from the blood of patients with metastatic cancers. In the lab, we apply microfluidics technology to human and mouse blood specimens, and adopt next-generation sequencing, molecular and computational biology, CTC cultures as well as loss of function screenings in xenograft models. Together, our approach aims to gain fundamental insights into the biology of CTC-clusters, and to identify novel therapeutic targets to suppress the metastatic spread of cancer.

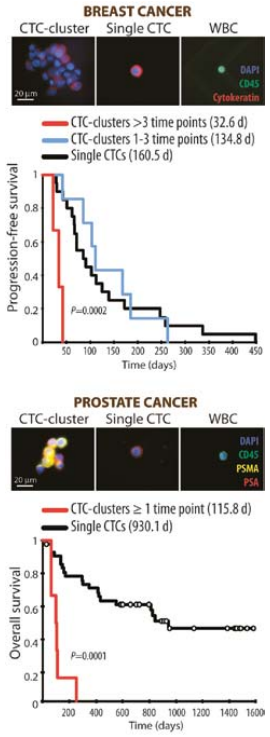

Fig. 1: The presence of CTC-clusters in patients with cancer correlates with poor prognosis. Kaplan-Meier analysis of patient data showing that the presence of CTC-clusters correlates with reduced progression-free survival and overall survival in patients with breast *(top)* and prostate cancer *(bottom)*, respectively.

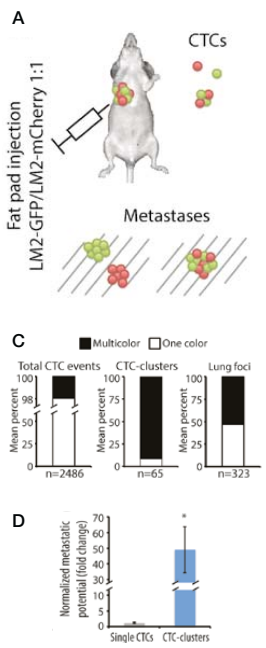

Fig. 2: CTC-clusters as metastatic precursors. **A)** Schematic of the experiment leading to spontaneous formation of multicolor CTC-clusters vs monocolor single CTCs from a primary breast tumor. **B)** Immunofluorescence images of CTCs *(left)* and immunohistochemistry staining of metastatic foci *(right)*. **C)** Bar graphs showing that the vast majority of CTC-clusters is multicolor and gives rise to multicolor metastatic foci. **D)** Bar graphs showing that CTC-clusters are up to 50-fold more metastatic than single CTCs.

Selected Publications

Gkountela S, Szczerba B, Donato C, Aceto N. (2016) Recent advances in the biology of human circulating tumor cells and metastasis. ESMO Open – Cancer Horizons. In press

Sarioglu AF*, Aceto N*, Kojic N, Donaldson, MC, Zeinali M, Hamza B, Engstrom A, Zhu H, Sundaresan TK, Miyamoto DT, *et al.* (2015) A microfluidic device for label-free, physical capture of circulating tumor cell clusters. Nat Methods 12, 685–691. *Equal contribution

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, *et al.* (2014) Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell 158, 1110–1122

Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, *et al.* (2014) Cancer therapy. *Ex vivo* culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science 345, 216–220

Tao JJ, Castel P, Radosevic-Robin N, Elkabets M, Auricchio N, Aceto N, Weitsman G, Barber P, Vojnovic B, Ellis H, *et al.* (2014) Antagonism of EGFR and HER3 enhances the response to inhibitors of the PI3K-Akt pathway in triple-negative breast cancer. Sci Signal 7, ra29

Connection to Clinical Practice

Prof. Dr. Christoph Rochlitz, Prof. Dr. Viola Heinzelmann, Prof. Dr. Alfred Zippelius, Prof. Dr. Walter Weber, Dr. Marcus Vetter, Dr. Sacha Rothschild, Dr. Julia Landin
University Hospital Basel

Isolating circulating tumor cells from the blood of cancer patients

The analysis of circulating tumor cells (CTCs) is an exceptional opportunity to study the biology of human cancer metastasis from minimally invasive biopsies, i.e. blood samples. In collaboration with Prof. Christoph Rochlitz, Prof. Viola Heinzelmann, Prof. Alfred Zippelius, Prof. Walter Weber, Dr. Marcus Vetter, Dr. Sacha Rothschild and Dr. Julia Landin at the University Hospital Basel, we routinely isolate and characterize CTCs from a variety of patients with metastatic cancers (e.g. breast, ovarian and lung cancer). Upon isolating CTCs from blood specimens with microfluidics technology, we process them for single cell resolution sequencing of their genome and transcriptome, to gain insights into the metastatic process. Further, we have implemented a protocol for deriving primary cultures from human CTCs, and use these as a model to study individualized drug susceptibility (i.e. so-called personalized medicine), as well as to study the requirement of specific genes for the metastatic process in xenograft models. With our approach, we aim to establish state-of-the-art and clinically relevant tools that will enable the identification of key vulnerabilities of cancer cells during the metastatic process.